Pharmacokinetics of Intravesical Mitomycin C in Superficial Bladder Cancer Patients

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ABSTRACT

Intravesical mitomycin C (MMC) therapy is used to treat superficial bladder cancer. This study was to establish the intra- and intersubject variabilities in the systemic (plasma) and target site (bladder) exposure to the drug and to identify the factors which contribute to these variabilities. The pharmacokinetics of MMC were studied in 10 patients. Treatment consisted of transurethral tumor resection followed by six weekly intravesical treatments with MMC (20 mg in 40 ml of water). The dosing solution was maintained in the bladder for 2 h. Pharmacokinetic studies were performed at the time of the first, fourth, and sixth or first, second, and fourth treatments with MMC for a total of 28 treatments. Concentration-time profiles of the plasma and bladder contents (i.e., urine), urine volumes, and urine pH were determined during and for up to 4 h after intravesical administration. Maximal plasma MMC concentrations averaged 43 ng/ml (range, 2.1-180.5 ng/ml) in treatment 1. In comparison, the MMC plasma concentration for myelosuppression reported in the literature is 400 ng/ml. Maximal plasma concentrations in treatments 2, 4, and 6 were at least 4-fold lower than those in treatment 1 and in most cases were below the detection limit of 0.5 ng/ml. This indicates that the absorption of MMC during the later treatments was less than in the first treatment given shortly after surgery. Urinary MMC concentrations during instillation declined from 519.4 ± 34.8 ng/ml (mean ± SD) in the dosing solution to 64.6 ± 39.4 ng/ml 2 h after instillation. Thus, the superfi-cial bladder tissue was exposed to drug concentrations 300- to >34,000-fold higher than the plasma-perfused systemic tissues. Intravesical exposure to MMC, as determined by the area under the urine concentration-time curve, showed large intra- and intersubject variabilities (range, 2.185-40.411 μg-min/ml). Pharmacokinetic analysis showed that the bladder exposure to MMC inversely correlated with the residual urine volume at the time of drug administration (P < 0.001), the urine production rate (P = 0.05), and the rate of drug removal by degradation and absorption during therapy (P < 0.01). At the end of the 2-h treatment, recovery of MMC from the bladder instillate ranged from 1 to 100% and correlated with the urine pH at the time of removal (P < 0.001). At pH between 5 and 5.5, <30% of the dose was recovered. In vitro incubation of MMC in urine demonstrated a 44% decrease in MMC concentrations after 2 h at pH 5, as compared to <4% loss at pH 7. Therefore, degradation accounted for more than half of the drug loss at low pH. In conclusion, these data indicate the large target site specificity of intravesical MMC, the variable but insignificant systemic exposure to MMC, and the large variabilities in target site exposure to MMC. The data further demonstrate the effect of residual urine volume, urine production, and drug removal by degradation and absorption during bladder exposure to MMC.

INTRODUCTION

Transurethral tumor resection and intravesical chemotherapy are the most commonly used treatment modality for superficial bladder cancer (1-3). Approximately 50-80% of patients treated with transurethral resection alone will have recurrent tumors (1, 4). The goal of intravesical chemotherapy is to prevent the recurrence and stage progression of superficial bladder tumors following initial treatment. MMC is one of the most widely used chemotherapeutic agents for this purpose (1-3). Intravesical MMC therapy following resection has been shown to reduce the recurrence rate to approximately 10-50% (1, 4-6). The reasons for the 5-fold variability in patient response are unclear. Huland et al. (2) recently compared the duration and intensity of intravesical MMC and doxorubicin therapy with regard to tumor recurrence (2). Tumor recurrence rates after long-term (>3 years) and short-term (<5 months) intravesical therapy were not significantly different. These data indicate that factors other than the duration of treatment may affect therapeutic efficacy. Differences in chemosensitivity of the tumor to the selected chemotherapy, initial tumor burden, tumor stage, patient selection, evaluation criteria, or drug exposure at the tumor site may contribute to the variable response to intravesical chemotherapy.

The tumor cell populations presumed to cause tumor recurrence are nonresected tumor cells remaining in the bladder wall, tumor cells dislodged from the site of resection and implanted elsewhere in the urothelium, and initiated tumor cells existing in other portions of the bladder wall. The exposure of these target tumor cells to the chemotherapeutic agent and, hence, the therapeutic efficacy are determined by the concentration-time profile of the drug in the systemic circulation. Pharmacokinetics in plasma and urine are needed to define the therapeutic efficacy of intravesical therapy.

There are limited pharmacokinetic data concerning MMC during intravesical therapy (7-10). Previous studies have examined the plasma pharmacokinetics in patients and showed that intravesical MMC therapy gave low systemic concentrations (7, 8). Other studies (9, 10) have measured the recovery of MMC in the urine voided at the completion of intravesical therapy. These studies showed that the urinary concentrations and recovery of MMC after therapy were highly variable, that plasma concentrations of MMC were independent of urinary pH and urinary MMC concentrations, and that MMC absorption into the systemic circulation was not affected by prolonged or previous intravesical administrations or by transurethral resection. However, these studies (9, 10) examined only MMC concentrations in the urine voided after therapy and did not study the pharmacokinetics in the urine. Furthermore, the first MMC treatment in these studies was at 2 weeks after surgery (10). To gain greater insight into the factors that determine the

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The abbreviations used are: MMC, mitomycin C; HPLC, high pressure liquid chromatography; AUC, area under the drug concentration-time profile; Vur, volume of urine remaining in the bladder at the time of instillation; AUCuary, AUC in the urine; AUCplas, AUC in the plasma; Vary, volume of the bladder contents at any time during instillation; kdes, rate constant describing absorption during instillation; kdes, rate constant describing the disappearance of drug from the bladder contents due to degradation, metabolism, and urine binding; kram, rate constant describing urinary production during instillation; PF, porfiromycin; tmax, end of sampling.

3 The abbreviations used are: MMC, mitomycin C; HPLC, high pressure liquid chromatography; AUC, area under the drug concentration-time profile; Vur, volume of urine remaining in the bladder at the time of instillation; AUCarily, AUC in the urine; AUCplas, AUC in the plasma; Vary, volume of the bladder contents at any time during instillation; kdes, rate constant describing absorption during instillation; kdes, rate constant describing the disappearance of drug from the bladder contents due to degradation, metabolism, and urine binding; kram, rate constant describing urinary production during instillation; PF, porfiromycin; tmax, end of sampling.
target site exposure and, therefore, affect the treatment outcome, it is necessary to establish the pharmacokinetic profiles in urine. Furthermore, the effect of surgery on MMC absorption is of interest. To our knowledge, there have been no studies conducted to examine the concentration-time profile of MMC in urine and the factors which control target site and systemic exposure during therapy. The present study was designed to (a) determine the MMC pharmacokinetics in the plasma and urine of patients during intravesical therapy, (b) determine the intra- and interindividual variability in pharmacokinetics in the plasma and urine, (c) determine the physiological and physicochemical source(s) of the variability, and (d) identify the treatment conditions which determine bladder exposure to MMC. These treatment conditions may be optimized to enhance the therapeutic efficacy of intravesical MMC therapy.

MATERIALS AND METHODS

Chemicals and Equipment. MMC (20-mg vials) was purchased from Bristol-Myers Co. (Wallingford, CT). The internal standard, PFM, was a gift from American Cyanamid Co. (Pearl River, NY). All other chemicals and solvents were of analytical grade and were obtained from Fisher Scientific (Cincinnati, OH). HPLC analysis showed that MMC and PFM were >99% pure. Both compounds were used as obtained.

HPLC analysis was performed using an Applied Biosystems model 400 pump (Applied Biosystems, Foster City, CA), a Waters 710B automated sampler, a Waters 440 UV detector with 365- and 216-nm filters or a Waters 490 UV detector operating at 365 and 216 nm (Waters Assoc., Milford, MA), and two Hewlett-Packard model 3390 integrators (Hewlett-Packard, Palo Alto, CA). In vitro drug stability studies were performed in a well-stirred water bath maintained at 37°C on a Whatman Dataplate model 440 (Whatman Ltd., Springfield Mill, England).

Patient Protocol. Patients were undergoing treatment for superficial bladder tumors. Therapy consisted of complete transurethral resection of all visible tumor and random cold cup biopsies followed by six weekly treatments with intravesical MMC. Table 1 shows the pertinent patient information. Patients were divided into two groups based on the pharmacokinetic study protocol. In group 1 (patients 1-4), pharmacokinetic studies were performed at the time of the first, fourth, and sixth treatments with MMC. The first intravesical treatment was administered between 1 and 58 days following surgery. Information gathered from group 1 patients was utilized to design the pharmacokinetic protocol for group 2 (patients 5-10). Pharmacokinetic studies on group 2 patients were performed at the time of the first, second, and fourth treatments with MMC. Group 2 patients received the first intravesical treatment 1-3 days following surgery, when postoperative hematuria had resolved.

Prior to the administration of MMC, patients were placed in a supine position and the bladder was emptied via a Foley catheter. MMC (about 20 mg in 40 ml of water) was instilled in the bladder via the catheter. The void volume of the catheter (size, 22-24 French) was 5 ml. Five to 10 ml of air was then flushed through the catheter to deliver the MMC solution remaining in the catheter. The catheter was clamped shut and the MMC-dosing solution was maintained in the bladder for 2 h. Patients in group 2 were instructed to roll over 90 degrees every 30 min. This procedure was to assure distribution and complete mixing of the dosing solution throughout the bladder. Serial blood and urine samples were obtained during therapy and for up to 6 h. Blood samples (10 ml each) were obtained through a “heparin lock” placed in an arm or jugular vein. Samples of the bladder content were obtained by connecting a 60-ml syringe to the outlet of the Foley catheter and slowly withdrawing the air and 5-10 ml of the bladder content to fill the catheter and syringe. The latex catheter was then pierced with a 1-ml syringe (30-gauge needle) and samples (0.1 ml each) were withdrawn. The catheter was flushed with air to deliver the contents of the catheter back into the bladder. By this method, the collection of the urine was completed within 2 min, thus minimizing the time of the MMC-dosing solution outside the bladder. Furthermore, by keeping the bladder content within the closed system, the possibility for bacterial contamination was minimized. The total volume of the samples was <1% of the content in the bladder. At the end of the 2-h instillation period, the contents of the bladder were withdrawn through the Foley catheter, and their volume and pH were immediately determined. After removal of the dosing solution, the bladder was emptied via the urethral catheter at 1-h intervals for up to 6 h. Blood samples were centrifuged at 1100 × g for 10 min at 4°C, and the plasma layer was transferred into glass tubes. The biological samples and aliquots of the dosing solution were stored at −20°C until analysis.

MMC Stability in Vitro. The stability of MMC in urine and buffers and the effect of pH were assessed in vitro. Urine was obtained from healthy male volunteers. MMC (final concentration, 125 µg/ml) was diluted in pH-adjusted urine, 0.25 M sodium acetate or 0.25 M potassium phosphate buffer at pH 5, 6, and 7. Triplicate samples were incubated for up to 8 h in a shaking water bath maintained at 37°C. Aliquots (100 µl) were withdrawn from each sample periodically, diluted 9-fold in water, and immediately analyzed by HPLC.

Sample Extraction and HPLC Analysis. Extraction and HPLC analysis of plasma samples were performed as previously described (11). Briefly, MMC and the internal standard, PFM, were extracted from 1 ml of buffered plasma with ethyl acetate. The organic extracts were evaporated under a stream of nitrogen, dissolved in aqueous mobile phase, and analyzed by HPLC. The lower detection limit of this assay was 0.5 ng/ml in plasma. Urine samples were diluted 9-fold in water and PFM was added. The diluted urine samples were directly injected into the HPLC. MMC and PFM were separated by isocratic reversed phase HPLC and UV detection at 365 and 313 nm. The stationary phase was a reversed phase C18 column (Pecosphere, 83 x 4.6 mm, 3-µm particle size; Perkin-Elmer, Norwalk, CT). The aqueous mobile phase contained 12.5% acetonitrile and 2.5 mM potassium phosphate buffer, adjusted to pH 6.9 with 2% phosphoric acid. The solvent flow rate was maintained at 1.5 ml/min. All analyses were performed at ambient temperature.

Data Analysis. The plasma concentration-time profiles were analyzed utilizing model-independent pharmacokinetic methods. The terminal slope of the ln (concentration) versus time plot following removal of the dose solution at 2 h was calculated using linear least squares regression. The AUC in plasma during the sampling period was calculated by the linear trapezoidal rule. The AUC from tamin to time infinity was calculated as the concentration at tamin divided by the absolute value of the terminal slope. The total AUC from time zero to infinity was the sum of these AUC values (12).

The concentration-time profiles of MMC in urine during instillation were analyzed according to the following model. The urine concentration of MMC, Cn, at any time, t, during intravesical administration was described by the following equations.

\[ C_n = \frac{Dose}{V_o} e^{-\beta t} + C_{res} \]  

and

\[ V_o = V_s + k_d + V_{res} \]  

where \( V_o \) is the volume of the urine at time, t; \( V_s \) is the volume of the MMC-dosing solution (40 ml); \( k_d \) is the zero-order rate constant describing urine production; \( V_{res} \) is the theoretical volume of residual urine present in the bladder at the time of instillation; \( k_d \) is the first-order rate constant describing absorption into the systemic circulation; and \( k_d \) is a hybridized first-order rate constant describing degradation, metabolism, and tissue binding. The derivation of Equation A is shown in the Appendix. Previous studies with MMC have shown that <10% of an i.v. dose is excreted into the urine (13). In this study, systemic blood concentrations of MMC were at least 300-fold less than the urine concentration. Hence, the transfer of MMC from the systemic circula-
Regional therapy to the tumor-bearing organs offers the therapeutic advantage of enhanced drug exposure of tumor cells while minimizing systemic drug concentrations and exposure of the systemic host tissues. The target site specificity of intravesical treatment was calculated as the ratio of bladder exposure (AUC bladder) to systemic exposure (AUC plasma). The extent of systemic absorption of MMC was calculated as the product of AUC plasma and the plasma clearance of MMC, obtained from the literature, divided by the intravesical dose (12). When plasma MMC concentrations of MMC were below the assay detection limit (<0.5 ng/ml), the systemic absorption of MMC was calculated as the plasma concentration at steady state (0.5 ng/ml) multiplied by the literature value for plasma MMC clearance and instillation time, divided by the intravesical dose (12).

Statistical analyses were performed using parametric and nonparametric methods. Pearson’s sample correlation is a parametric test which identifies linear relationships between variables (14). Spearman’s rank correlation is a nonparametric test which identifies both linear and nonlinear relationships (14). Correlations between variables were examined at a 5% level of significance. P values are reported for Spearman’s rank correlation coefficient. Differences between group 1 and 2 patients were determined using a single-factor analysis of variance.

RESULTS

Pharmacokinetics in Plasma. In 21 of the 28 studies, plasma concentrations were below the assay detection limit (0.5 ng/ml) for most or all of the time points sampled. Complete plasma MMC concentration-time profiles were obtained in the 7 remaining intravesical treatments. Initial pharmacokinetic studies in group 1 patients were performed at the time of the first, fourth, and sixth intravesical MMC treatments. Data from these studies suggested a relationship between the maximal plasma MMC concentration and the duration between surgery and treatment, with plasma concentrations below the limit of detection in 7 of 8 treatments administered >2 days after surgery (Table 1). To further determine the time dependence of systemic MMC absorption, pharmacokinetic studies in group 2 patients were performed at the time of the first, second, and fourth intravesical MMC treatments. In addition, the first intravesical treatment in all group 2 patients was administered 1–3 days following surgery, as soon as postoperative hematuria had resolved. Maximal plasma MMC concentrations and the AUC plasma again correlated with the length of time between surgery and intravesical treatment. In group 2 patients, MMC was detectable in plasma of all patients during the first intravesical treatment, with mean maximal plasma MMC concentrations of 50.0 ng/ml (Table 1). A week later, MMC was detectable in only 2 of 6 patients with a mean maximal concentration of 3.7 ng/ml. At the time of treatment 4, MMC was detected in only one of 6 patients. The peak MMC concentration during treatments given 1–3 days after surgery averaged 43 ng/ml, which was significantly higher (P < 0.005) than the mean peak plasma concentration during treatments administered 1 week or more after surgery. In contrast, van Helsdingen et al. (10) reported plasma concentrations of about 6 ng/ml up to 8 weeks after tumor resection. Lower plasma concentrations in our study may be attributed to a smaller intravesical dose (20 mg of MMC in 40 ml) compared to that used by van Helsdingen et al. (10) (40 mg of MMC in 40 ml).

Table 1: Patient information and plasma pharmacokinetic data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Treatment</th>
<th>Time since surgery (days)</th>
<th>No. of tumors</th>
<th>Tumor size (cm) and biopsies</th>
<th>Tumor grade/stage</th>
<th>Cmax (ng/ml)</th>
<th>AUCplasma (ng-min/ml)</th>
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<td>2</td>
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<td>4</td>
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<tr>
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<tr>
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<tr>
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<td>6</td>
<td>8</td>
<td>3</td>
<td>biopsies</td>
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<tr>
<td></td>
<td>7</td>
<td>24</td>
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<tr>
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<td>2</td>
<td>5–6 mm (2)</td>
<td>III/T1</td>
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<td>2</td>
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<td>biopsies</td>
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<td>8</td>
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<td>2</td>
<td>biopsies</td>
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</table>
Plasma concentrations increased rapidly following intravesical administration and were detectable within 10–30 min. After removal of the urine at 2 h, plasma MMC concentrations declined log linearly with time and had a half-life of 63 ± 11.5 min (mean ± SD, n = 5). This half-life was comparable to that reported in previous investigations of MMC disposition after i.v. administration (15, 16). The AUC<sub>plasma</sub> values in the 10 patients showed a 12-fold intersubject variability during the first treatment.

Pharmacokinetics in Urine. Complete MMC concentration-time profiles in urine were obtained for 27 of the 28 treatments. Fig. 1 shows the average urine concentration-time profiles for patients given an intravesical dose of MMC on the first, second, fourth, and sixth weeks following surgery. The concentration at the zero time point was the mean concentration of the dosing solution prior to instillation. All other data points were the mean drug concentrations in urine. The solid line represents a computer-simulated urine concentration-time profile, using mean values for the pharmacokinetic parameters, (k<sub>a</sub> + k<sub>d</sub>, k<sub>0</sub>, and V<sub>ren</sub>), and for each weekly treatment.

In group 1 patients, MMC concentrations in urine declined from 405 ± 63.2 μg/ml (range, 347–517 μg/ml) in the dosing solution to 215 ± 78.5 μg/ml (range, 106–326 μg/ml) after 5 min in the bladder and to 42.3 ± 32.4 μg/ml (range, 1–94 μg/ml) after the 2-h instillation period. MMC concentrations in urine declined because of several factors, i.e., dilution by residual urine present in the bladder at the time of instillation, production of additional urine during the 2-h treatment, and removal by absorption, degradation, metabolism, and tissue binding. The pharmacokinetic parameters describing these processes, i.e., residual volume (V<sub>ren</sub>), rate of urine production (k<sub>0</sub>), and rate constants of absorption and degradation (k<sub>a</sub> + k<sub>d</sub>), were calculated from the urine concentration-time profiles for each of the 27 treatments. Data are shown in Table 2. In group 1, V<sub>ren</sub> ranged from 0 to 76 ml. This volume is relatively large compared to the 40-ml volume of the dosing solution. The V<sub>ren</sub> term accounted for the 47% decrease of MMC concentrations within 5 min. The sum of the first-order absorption and degradation rate constants, (k<sub>a</sub> + k<sub>d</sub>), was 0.0146 ± 0.0068/min in group 1. This was primarily due to degradation and/or metabolism. There was no significant drug loss due to absorption based on the low plasma MMC concentrations. The urine production rate, k<sub>0</sub>, was 1.48 ± 1.25 ml/min.

In order to minimize the effects of residual urine on the intravesical exposure to MMC in group 2 patients, the positions of the patient and Foley catheter were adjusted several times in an attempt to more completely drain the bladder prior to instillation of the dosing solution. In addition, patients were instructed to roll over 90 degrees during therapy. This procedure was to assure distribution of the dosing solution throughout the bladder and complete mixing with urine present within the bladder. In group 2 patients, MMC concentrations in urine declined from 519 ± 34.8 μg/ml (range, 457–570 μg/ml) in the dosing solution to 315 ± 127 μg/ml (range, 107–536 μg/ml) after 5 min and to 64.6 ± 39.4 μg/ml (range, 11–152 μg/ml) after the 2-h instillation period. Table 2 shows the computer-fitted pharmacokinetic parameters for group 2. V<sub>ren</sub>, ranged from 0 to 131 ml and again showed large intra- and intersubject variability. (k<sub>a</sub> + k<sub>d</sub>) was 0.0144 ± 0.0211/min and k<sub>0</sub> was 1.48 ± 1.07 ml/min. Values for V<sub>ren</sub> (k<sub>a</sub> + k<sub>d</sub>), and k<sub>0</sub> in group 2 patients were not significantly different from those of group 1 (P > 0.9).

The target site specificity of intravesical therapy was calculated as the ratio of AUC<sub>bladder</sub> to AUC<sub>plasma</sub>. The AUC<sub>bladder</sub> averaged 9,677 ± 3,594 μg-min/ml in group 1 and 17,046 ± 10,832 μg-min/ml in group 2. In 7 of 28 treatments, the plasma MMC concentration-time profile permitted the estimation of the terminal slope and, hence, the calculation of the AUC<sub>plasma</sub>. The AUC<sub>plasma</sub> averaged 2,999 ng-min/ml in group 1 (n = 2) and 7,847 ng-min/ml in group 2 (n = 5). In these treatments, the target site specificity of intravesical therapy ranged from 300 to >34,000 (n = 7). In the remaining 21 treatments, the plasma concentrations were not detectable (<0.5 ng/ml) and, hence, the ratio of AUC<sub>bladder</sub> to AUC<sub>plasma</sub> approached infinity. These data demonstrate the large target site specificity of intravesical MMC therapy.

Bladder exposure would be expected to vary as a function of absorption, degradation, metabolism, and/or binding (k<sub>a</sub> + k<sub>d</sub>), residual urine volume (V<sub>ren</sub>), and urine production (k<sub>0</sub>) during therapy, in accordance with Equations A and B. Intra- and intersubject variability was 18-fold in the AUC<sub>bladder</sub>, with values ranging from 2,185 to 40,411 μg-min/ml (Table 3). AUC<sub>bladder</sub> correlated with (k<sub>a</sub> + k<sub>d</sub>), V<sub>ren</sub>, and k<sub>0</sub> (P < 0.01, P < 0.001, and P = 0.05, respectively) (Fig. 2). These data indicate that variable first-order disposition, residual urine present in the bladder at the time of instillation, and urine production during instillation were primarily responsible for the intra- and intersubject differences in bladder exposure.

Recovery of MMC from the Bladder. At the end of the 2-h treatment, the bladder was emptied via the Foley catheter. The volume of urine removed at 2 h ranged from 48 to 570 ml. The amount of MMC recovered ranged from 1 to 100% of the dose (Table 2). The average recoveries were 37.2 ± 29.9% in group 1 patients and 57.9 ± 29.0% in group 2 patients. The interindividual variation was between 0.5- and 4-fold. Intraindividual variation, in 8 of 10 cases, was <2-fold and in the two remaining cases was 3- to 4-fold. The variability appeared to be random and was not patient specific. The recovery of MMC in urine voids from 2–6 h (up to 4 h after removal of the dose) was insignificant (≤7%) in 27 of 28 cases, and in the remaining case the recovery was 34%.
The concentration-time profiles of MMC in the bladder content during intravesical therapy were computed fitted to Equations A and B. The $V_{\text{tris}}$, $k_a$, and the $(k_a + k_d)$ were determined. $\text{AUC}_{\text{bladder}}$ was calculated by the linear trapezoidal rule. The fraction of the dose recovered was calculated as the amount recovered via the Foley catheter divided by amount administered. NA, not applicable.

### Table 2 Pharmacokinetics of MMC in urine

<table>
<thead>
<tr>
<th>Patient</th>
<th>Treatment</th>
<th>Group</th>
<th>$k_a + k_d$ (1/min)</th>
<th>$k_a$ (ml/min)</th>
<th>$V_{\text{tris}}$ (ml)</th>
<th>$\text{AUC}_{\text{bladder}}$ (ug·min/ml)</th>
<th>pH</th>
<th>% dose recovered at 2 h</th>
<th>% dose recovered from 2-6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1.04 x 10^-5</td>
<td>0.60</td>
<td>67.4</td>
<td>10,826</td>
<td>7.2</td>
<td>32</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1.64 x 10^-5</td>
<td>0.92</td>
<td>62.8</td>
<td>5,584</td>
<td>6.0</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>4</td>
<td>1.97 x 10^-5</td>
<td>1.06</td>
<td>76.3</td>
<td>6,299</td>
<td>5.9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>2</td>
<td>6</td>
<td>2.02 x 10^-5</td>
<td>1.75</td>
<td>47.7</td>
<td>5,962</td>
<td>7.0</td>
<td>55</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>3</td>
<td>7.90 x 10^-5</td>
<td>1.64</td>
<td>15,072</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>4</td>
<td>9.81 x 10^-5</td>
<td>4.61</td>
<td>37.7</td>
<td>11,084</td>
<td>6.8</td>
<td>70</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1</td>
<td>7.37 x 10^-5</td>
<td>0.63</td>
<td>8.8</td>
<td>14,744</td>
<td>5.2</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>4</td>
<td>2.38 x 10^-5</td>
<td>1.33</td>
<td>0</td>
<td>7,857</td>
<td>5.0</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>6</td>
<td>2.02 x 10^-5</td>
<td>0.69</td>
<td>3.2</td>
<td>9,667</td>
<td>6.0</td>
<td>31</td>
<td>0</td>
</tr>
</tbody>
</table>

### Table 3 Intra- and intersubject variability in target site exposure

The concentration-time profiles of MMC in the bladder content during intravesical therapy were computed fitted to Equations A and B. $\text{AUC}_{\text{bladder}}$ was calculated by the linear trapezoidal rule. NA, not applicable.

<table>
<thead>
<tr>
<th>Number of treatments</th>
<th>$\text{AUC}_{\text{bladder}}$ (ug·min/ml)</th>
<th>Coefficient of variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-subject variability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>10,783 ± 4.924*</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>20,983 ± 11,345</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>16,058 ± 11,610</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>8,816 ± 2,541</td>
</tr>
<tr>
<td>Intra-subject variability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>10,826*</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>5,948 ± 358</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>13,078 ± 3,988*</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>10,756 ± 3,570</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>17,808 ± 2,443</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>16,524 ± 20,800</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>22,225 ± 15,949</td>
</tr>
<tr>
<td>8</td>
<td>3</td>
<td>10,386 ± 2,561</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>17,243 ± 9,883</td>
</tr>
<tr>
<td>10</td>
<td>3</td>
<td>18,062 ± 9,506</td>
</tr>
</tbody>
</table>

* Mean ± SD.

* Single determination.

* NA*, not applicable.

* Average ± range.

The recovery of MMC at 2 h increased with increasing urine pH at the time of removal (Fig. 3; $n = 28$, $r = 0.658$, $P < 0.001$). At a pH between 5 and 5.5, <30% of the dose was recovered. At pH 6.5-8, >80% of the dose was recovered. Several factors may have accounted for this correlation and the incomplete recovery of the dose: (a) pH-dependent absorption process, (b) pH-dependent binding to bladder wall tissue, (c) incomplete withdrawal of urine at 2 h, (d) enzymatic metabolism/degradation of MMC by the bladder wall, (e) degradation of MMC in urine, and/or (f) pH-dependent binding to the Foley catheter. Plasma MMC levels in this patient group indicated that systemic absorption of MMC was minimal. Therefore, a pH-dependent absorption process was unlikely to contribute significantly to the disappearance of MMC during instillation. The binding of MMC to bladder wall tissue was unlikely to have contributed significantly. In an independent study, we found that the average bladder wall concentration ranged from 0.5 to 58 μg/g of wet tissue weight in dogs receiving the same dose of MMC (20 mg in 40 ml of water instilled for 2 h) (17). These concentrations were equivalent to <500 μg or 3% of the intravesical dose/8 g of bladder tissue. Incomplete withdrawal of the bladder contents would be expected to occur randomly, independently of pH. Furthermore, urine voids taken at 1, 2, and 4 h following removal of the instillate contained an insufficient amount of MMC to account for the low recovery in 27 of 28 cases (Table 2). Incomplete withdrawal of urine may be a function of the inability to completely void the bladder because of physiological conditions or positioning of the urethral catheter. In this case, the recovery may be related to $V_{\text{tris}}$ in individual treatments. However, there was no apparent correlation between $V_{\text{tris}}$ and the presence of MMC in the later urine voids, suggesting that other factors are primarily responsible for the pH-dependent drug loss. Enzymatic metabolism/degradation of MMC by the bladder wall may have a significant effect. Previous investigations have shown that the urinary bladder in mice contain high activity of endogenous quinone reductase, an enzyme which metabolizes MMC (18). The significance of this process in patients is unknown.

**In Vitro Experiments.** As shown above, rate constants for first-order disposition ($k_a + k_d$) correlated with $\text{AUC}_{\text{bladder}}$. The significance of nonenzymatic degradation and binding to the Foley catheter to bladder exposure and the recovery of MMC
were studied under in vitro conditions. Nonenzymatic degradation of MMC in pH-adjusted urine and buffers at pH 5, 6, and 7 was pH dependent and occurred as a first-order process. Nonenzymatic degradation at pH 5 (half-life, 2.8 h) proceeded >3-fold faster than degradation at pH 6 (half-life, 12.2 h) and >12-fold faster than degradation at pH 7 (half-life, 38 h). A calculation based on these rate constants showed that 44% of the MMC dose would have been lost because nonenzymatic degradation in 2 h at pH 5. In patients, the average recovery at pH 5-5.5 was 26.3 ± 12.9%, equivalent to a loss of 72.7 ± 12.9% (n = 4). Hence, a loss of 30% of the dose was not accounted for. These data show that nonenzymatic degradation contributed significantly to decreased bladder exposure and the pH-dependent loss of MMC but that it was not the sole determinant of these parameters.

Binding of MMC to the Foley catheter as a function of pH was also studied in vitro. The binding of MMC to the latex catheter was <1% at pH 5 and 7 in urine and buffers. This process, therefore, did not contribute to the low recovery of MMC at acidic pH.

DISCUSSION

The rationale for intravesical therapy is to expose tumor cells located in the bladder wall to concentrations much higher than those achieved systemically during treatment. Target site drug exposure is an important determinant of treatment efficacy, while systemic exposure is an important determinant of host toxicity. Previous studies have focused on the concentration-time profiles of intravesical agents in the systemic circulation during therapy and have ignored the pharmacokinetics at the target site. The exposure of tumor cells located in the bladder wall is highly dependent on the AUC bladder and the kinetics of drug penetration through the bladder wall. Tumor response to chemotherapy will depend on the intravesical exposure and the inherent chemosensitivity of the tumor to the drug. Intravesical MMC therapy has been shown to reduce the tumor recurrence rate to approximately 10–50% (1–6). Questions concerning the relative contributions of pharmacokinetics, tissue penetration, and chemosensitivity to the highly variable patient response remain unanswered. Our pharmacokinetic data demonstrate large intra- and intersubject variability in the target site exposure to MMC during intravesical therapy. The present study is the first to describe urinary MMC concentration-time profiles during intravesical therapy, to identify the inter- and intrasubject variability in the target site exposure, and to evaluate the factors which influence the target site exposure. Concurrent studies in our laboratories address the variability of drug penetration into the bladder tissue and tumor chemosensitivity (17, 19, 20).

Target site exposure is of great concern in achieving optimal intravesical therapy, because tumor cells located in the bladder wall must receive effective concentrations. The target site specificity of intravesical therapy was substantial. Ratios of AUC bladder to AUC plasma averaged >6,000 in treatments administered 1–3 days following surgery and approached infinity in subsequent treatments. Our data show that tumor cells located in the bladder urothelium and directly accessible by MMC in urine received concentrations several orders of magnitude higher than the systemic tissues. However, these tumor cells received variable drug exposure from treatment to treatment and from patient to patient. Because the drug concentration in the bladder contents is the driving force for drug absorption across the urothelium and penetration into deeper bladder wall tissue, the same variability would apply to tumor cells located in the lamina propria or muscle layers. The penetration of MMC in bladder wall was studied in dogs and cystectomy patients (17, 20). Urine pharmacokinetic data, combined with tissue penetration and chemosensitivity data (17, 19, 20), showed that the therapeutic outcome of intravesical therapy is largely dependent on the MMC concentration in urine. The variable AUC bladder may partially account for the variability in patient response to intravesical MMC. A detailed discussion is given in a separate publication (17).

The AUC bladder showed an 18-fold intra- and intersubject variability during therapy. Residual urine present in the bladder at the time of instillation and nonenzymatic degradation at
acids were the primary causes of the different AUC bladder. Intra- and interindividual differences in metabolism and/or absorption may have also contributed to the large variability. Incomplete bladder emptying via urethral catheters and residual urine volumes averaging 130–200 ml have been previously reported in patients (21). Our data showed $V_{\text{ur}}$ values from 0 to 130 ml. The cause of the intra- and interindividual variation in bladder emptying is unknown. Systemic absorption of MMC could not account for the substantial variability in AUC bladder because plasma concentrations did not correlate with urine concentrations. Furthermore, the low plasma concentrations indicate that insignificant amounts of MMC reached the systemic circulation. Using a literature value of 10 ml/min/kg for the plasma clearance of MMC (16), we estimated that on average <6% of the intravesical dose was absorbed into the systemic circulation. In comparison, the fraction of the MMC dose lost after 2 h averaged 52%. Hence, systemic absorption accounted for only a small component of the loss of MMC. Concurrent studies in our laboratories demonstrated that the amount of MMC absorbed in the bladder wall of dogs and patients during intravesical therapy is also insufficient to account for the low recovery (17, 20). Hence, there are other processes, in addition to absorption, which removed MMC from urine, as discussed below.

It should be noted that, in spite of the considerable variability in bladder pharmacokinetics, systemic exposure to MMC was minimal and below the threshold for toxicity. MMC plasma levels in excess of 400 ng/ml have been associated with myelosuppression (22). In our studies, the highest plasma concentration achieved in 28 treatments with MMC was 180 ng/ml, while plasma concentrations in 16 of 28 treatments were <0.5 ng/ml. These data demonstrate that intravesical MMC yields high concentrations in the bladder while avoiding systemic toxicity and confirm the advantage of intravesical therapy.

A previous study examined the relationship between the maximal plasma MMC concentrations and the MMC concentrations in urine recovered at the end of intravesical therapy and found no correlation (10). Single point determination of urine concentration at the end of therapy serves only as a measurement of recovery. A higher urine concentration implies greater recovery, based on the assumptions that the MMC concentration in urine is the driving force for systemic absorption and that systemic absorption is the major mechanism of drug removal from the urine. However, this study (10) did not obtain paired urine and plasma concentrations, which are needed to estimate the transfer function between the bladder and systemic compartment and the physiological and physicochemical factors which determine target site and systemic exposure. Hence, it is not surprising that the MMC concentration in urine recovered at the end of therapy did not correlate with the peak plasma concentration. Furthermore, other drug removal processes in addition to absorption, i.e., degradation and dilution by urine, complicate the assessment of this relationship. In comparison, our study established the complete urine concentration-time profile during therapy. Pharmacokinetic analysis of these profiles, using Equations A and B, provided the values of $V_{\text{ur}}$, $k_a$, and $(k_a + k_d)$. Our analysis showed that $(k_a + k_d)$ did not correlate with the plasma MMC concentrations. However, target site exposure, i.e., AUC bladder, correlated with $(K_a + k_d)$, $V_{\text{ur}}$, and $k_0$.

The systemic MMC concentrations achieved from absorption of the intravesical dose were significantly higher in treatments administered shortly after surgery than in treatments given 1 or more weeks after surgery. These data indicate that the bladder urothelium was able to rapidly repair itself and its absorptive barrier function within 1–2 weeks following surgical resection. These data demonstrate that the timing of the first intravesical dose may be an important determinant of systemic toxicity during intravesical therapy. Previous studies showed that systemic toxicity and cystitis of thiopeta was lower in groups receiving intravesical treatment several weeks after surgery (6). The intravesical administration of bacillus Calmette-Guerin occasionally leads to systemic infection and sepsis (6). It may be more appropriate to administer bacillus Calmette-Guerin in 1–2 weeks after surgery, when the bladder urothelium has had time to heal. Data in Table 1 demonstrate that maximal plasma MMC levels did not correlate with the area of resection. For example, patient 7 had an area of resection twice as large as patient 5, but the maximal plasma concentrations in both patients were comparable. Conversely, patients 5 and 6 had a similar area of resection, but the maximal plasma concentration in patient 6 was >7-fold higher than that of patient 5. All resections extended to the deeper muscle layers. Therefore, while the surgical resection wound contributed to the intra- and intersubject variability in plasma pharmacokinetics, it was not the sole determinant. Other factors, such as variability in the concentration-time profile in urine and/or the systemic clearance of MMC, might have also affected the systemic pharmacokinetics.

A previous study suggested degradation of MMC in urine, substantial loss of MMC in the catheter, and/or absorption into the urothelial wall and subsequent metabolic degradation as explanations for the low recovery of MMC after intravesical therapy (10). Our in vitro studies ruled out loss of MMC in the catheter and showed that the rate of nonenzymatic degradation of MMC was 12-fold slower at pH 7 relative to pH 5. It would be logical to buffer the bladder instillate to pH 7 during therapy in order to decrease the rate of nonenzymatic degradation and thereby increase bladder exposure. This approach must be carefully considered in light of the pH-dependent activity of MMC. Kennedy et al. (23, 24) demonstrated enhanced in vitro activity of MMC in EMT6 cells under acidic pH and hypoxic conditions. Recent studies suggest that the pH-dependent activity of MMC is related to its enzymatic activation (24–26). NADPH:cytochrome P-450 reductase, NAD(P)H: (quinone-acceptor) oxidoreductase, and xanthine oxidase have been implicated in the activation and/or detoxification of MMC under various in vitro conditions (27–30). Therefore, the selection of the optimal pH for intravesical MMC therapy is a balance between nonenzymatic degradation and enzymatic activation. Further studies describing the distribution and roles of enzymes involved in MMC activation and detoxification within the bladder wall and superficial bladder tumors, as well as the relationship between pH and alkylating activity, are needed.

In summary, intravesical MMC therapy produced significant target site specificity for the treatment of superficial bladder cancer. Systemic exposure to MMC was well below the threshold for toxicity, while concentrations in the bladder were maintained several orders of magnitude higher. The bladder exposure to MMC demonstrated large intra- and intersubject variability. Residual urine present in the bladder at the time of instillation, the production of urine during therapy, and drug removal by absorption and degradation during therapy were the major determinants of the target site exposure to MMC, i.e., AUC bladder. Concurrent studies demonstrated the AUC bladder as an important determinant of drug concentration (and, hence,
the therapeutic efficacy) in the urothelium (T1 tumors), lamina propria (T1 tumors), and muscle layers (T2 tumors) (17, 19, 20). Further enhancement of target site exposure requires changes in the treatment protocol, including more complete bladder emptying, control of the urine pH for optimal drug activity and stability, decreased fluid intake prior to and during therapy, increased instillation periods, and more complete distribution and mixing of the dosing solution within the bladder during therapy.

ACKNOWLEDGMENTS

We thank Dr. John Nesbitt for his participation and identifying suitable patients for this study. The assistance of Dr. Donn Young in the statistical analysis is gratefully acknowledged.

APPENDIX

The derivation of Equation A is as follows. The following model describes the concentration-time profiles of MMC in the bladder contents.

\[
\begin{align*}
\frac{dA_u}{dt} & = -(k_a + k_d)A_u \\
A_u & = V_o \cdot C_u
\end{align*}
\]

By substitution,

\[
\frac{d(V_o \cdot C_u)}{dt} = -(k_a + k_d)V_o \cdot C_u
\]

By partial differentiation,

\[
C_u \left( \frac{dV_o}{dt} + V_o \left( \frac{dC_u}{dt} \right) \right) = -(k_a + k_d)V_o \cdot C_u
\]

where \( V_o = V_0 + k_d + V_{res} \).

Substituting the expression for \( V_o \) and rearranging,

\[
\frac{dC_u}{dt} = -C_u \left( \frac{d(V_0 + k_d + V_{res})}{dt} \right)
\]

\[
- (k_a + k_d)(V_0 + k_d + V_{res})C_u
\]

Rearranging,

\[
\frac{dC_u}{dt} = \frac{C_u[ -k_0 - (k_a + k_d)(V_0 + k_d + V_{res})]}{(V_0 + k_d + V_{res})}
\]

By integration,

\[
\ln \left( \frac{C_u}{C_0} \right) = \ln \left( \frac{V_0 + V_{res}}{V_0 + k_d + V_{res}} \right) - (k_a + k_d)t
\]

\[
C_u = \frac{C_0(V_0 + V_{res})}{V_0 + k_d + V_{res}} e^{-(k_a + k_d)t}
\]

\[
= \frac{\text{Dose}}{V_o} e^{-(k_a + k_d)t}
\]

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Pharmacokinetics of Intravesical Mitomycin C in Superficial Bladder Cancer Patients

James T. Dalton, M. Guillaume Wientjes, Robert A. Badalament, et al.


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